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Original article

Overexpression of microRNA-155 suppresses chemokine expression induced by Interleukin-13 in BEAS-2B human bronchial epithelial cells

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Abbreviations:
CCL, CC chemokine ligand; CXCL, C-X-C motif ligand; dsRNA, double-stranded RNA; ELISA, enzyme-linked immunosorbent assay; IL-13, interleukin-13; IRF-3, interferon regulatory factor 3; miR-155, microRNA-155; miRNA, microRNA; NF-κB, nuclear factor-kappa B; PCR, polymerase chain reaction; SEM, standard error of the mean; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-alpha; TLR3, Toll-like receptor 3; 3′UTR, 3′ untranslated regions

ABSTRACT

Background: MicroRNAs are non-coding small RNAs that regulate expression of target genes by binding to 3′ untranslated regions. In this study, we used bronchial epithelial cells to investigate *in vitro* the role of the microRNA miR-155 in the expression of chemokines associated with airway inflammation. miR-155 has previously been reported to regulate allergic inflammation.
Methods: BEAS-2B bronchial epithelial cells were cultured and transfected with mimic or inhibitor oligonucleotides to overexpress or downregulate miR-155, as confirmed by real-time PCR. Cells were then stimulated with tumor necrosis factor-alpha, interleukin-13 (IL-13), and a double stranded RNA that binds Toll-like receptor 3. Expression and secretion of the chemokines CCL5, CCL11, CCL26, CXCL8, and CXCL10 were then quantified by real-time PCR and ELISA, respectively. Phosphorylation of signal transducer and activator of transcription 6 (STAT6), a target of the IL-13 receptor, was analyzed by ELISA.
Results: miR-155 overexpression significantly suppressed IL-13-induced secretion of CCL11 and CCL26. These effects were specific, and were not observed for other chemokines, nor in cells with down-regulated miR-155. miR-155 overexpression also suppressed CCL11 and CCL26 mRNA, but did not affect expression of the IL-13 receptor or phosphorylation of STAT6.
Conclusions: miR-155 specifically inhibits IL-13-induced expression of eosinophilic chemokines CCL11 and CCL26 in bronchial epithelial cells, even though the 3'-untranslated region of these genes do not contain a consensus binding site for miR-155.

Introduction

microRNAs (miRNAs) are small non-coding RNAs of 18–25 nucleotides that regulate gene expression via a “seed” region, which specifically binds to the 3'-untranslated region of the target mRNA to suppress translation or induce degradation. Accumulating evidence suggests that miRNAs regulate an array of biological processes, including inflammation and allergic disease.[1]

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A growing body of research suggests that the microRNA miR-155 might play an important role in the pathogenesis of asthma,[2] a common airway disease characterized by damage, chronic inflammation, and hyperresponsiveness in the bronchial epithelia. For instance, Rodriguez *et al.*[3] reported that a defect in miR-155 in a mouse model causes airway inflammation and other pathological features that resemble asthma. Decreased expression of miR-155 has also been observed in the airway of asthmatic patients.[4–6] In addition, Martinez-Nunenz *et al.*[7] demonstrated that miR-155 directly targets interleukin-13 receptor alpha 1 and suppresses its expression in human macrophages. Finally, miR-155 inhibits the replication of human rhino virus, which frequently exacerbates asthma.[8] Thus, investigation of the clinical relevance of miRNAs to allergic and/or pulmonary diseases would advance diagnosis and therapy. Indeed, therapeutic approaches based on miRNA seem promising in light of the ability of specific miRNAs to modulate multiple molecular processes associated with disease pathogenesis.[9,10]

Chemokines from bronchial epithelial cells have also been reported to contribute to chronic airway inflammation by recruiting inflammatory cells. In particular, Larose *et al.*[11] recently reported that CC chemokine ligand 26 (CCL26) is abundantly expressed in the epithelium of patients with severe asthma. CCL26 and CCL11 are believed to be induced by key Th2 cytokines like interleukin-13 (IL-13) to elicit eosinophilic inflammation via CC chemokine receptor 3.[12,13] In addition, we have demonstrated that the cytokines tumor necrosis factor-alpha (TNF-α) and IL-13, as well as a double-stranded RNA (dsRNA) that binds Toll-like receptor 3, differentially regulate expression of the inflammatory chemokines CCL11/Eotaxin, CCL26/Eotaxin-3, CCL5/RANTES, CXCL8/IL-8, and CXCL10/IP-10 in bronchial epithelial cells.[12,14–16]

In this study, we characterized for the first time the role of miR-155 in the expression of inflammatory chemokines in bronchial epithelial cells, as well as the underlying molecular mechanisms.

Methods

Cell culture and reagents

The BEAS-2B human airway epithelial cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). This cell line was derived from non-tumor bronchial epithelial cells transformed with SV40.[17] Cells were grown at 37 °C in a humidified atmosphere with 5% CO_2, in Dulbecco's modified Eagle medium/F12 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 ng/mL streptomycin (Invitrogen, Tokyo, Japan). Recombinant human cytokines TNF-α and IL-13 were purchased from R & D Systems (Tokyo, Japan). An agonist of Toll-like receptor 3 (TLR3), synthetic dsRNA poly IC was obtained from Sigma-—Aldrich (Tokyo, Japan). Culture supernatant, RNA, and cell lysates were collected, and stored at −80 °C until experiments.

Chemokine secretion

The concentration of CCL11, CCL26, CCL5, CXCL8, and CXCL10 in the cell culture supernatant was measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) according to the manufacturer's protocol, and as described previously.[14,16] The ELISA plate reader Glomax-Multi Detection System (Promega, Tokyo, Japan) was used to measure absorbance.

mRNA expression

Total RNA was extracted from cell cultures, and transcribed with High-Capacity cDNA Archive Kit (Applied Biosystems, Tokyo, Japan). Abundance of CCL11, CCL26, CCL5, CXCL8, CXCL10, and IL-13 receptor alpha 1 mRNA was measured by real-time polymerase chain reaction (PCR) using TaqMan probes and Real-Time PCR System 7500 (Applied Biosystems, Tokyo, Japan), as described previously.[14,16]

Transfection of miR-155 inhibitor or mimic

miR-155 mimic (hsa-miR-155, mirVana™ miRNA mimic), miR-155 inhibitor (hsa-miR-155, mirVana™ miRNA inhibitor), and negative control miRNA were purchased from Ambion (Tokyo, Japan). The inhibitor is a pre-designed antisense oligonucleotide that downregulates miR-155. The mimic contains same sequence of endogenous miR-155 and can increase the expression level of miR-155. It is known to show same function as mature endogenous miR-155. Negative control miRNA is a non-specific oligonucleotide with a random sequence and without measurable effects. BEAS-2B cells were seeded in 6-well plates, cultured to 50% confluence, transfected with 25 nM miRNAs in 5 μL Lipofectamine 2000 (Promega, Tokyo, Japan), and grown for 24 h in Dulbecco's modified Eagle medium/F12 medium with 10% fetal bovine serum and without antibiotics. Media were then replaced with fresh media supplemented with 100 U/mL penicillin, and 100 ng/mL streptomycin. After another 24 h, cells were stimulated with cytokines or dsRNA. Finally, cells and supernatant were harvested 24 h after stimulation.

miR-155 expression

Total miRNA was extracted from cells using mirVana™ miRNA Isolation Kit (Ambion, Tokyo, Japan), following the manufacturer's protocol. Purified RNA (5 ng) was transcribed with TaqMan microRNA reverse transcription kit (Applied Biosystems) as described in the manufacturer's protocol, and amplified with a primer set for miR-155, using HY3 as endogenous control. Predesigned TaqMan probes for miR-155 and HY3 were purchased from Applied Biosystems. The probes are labeled with a FAM fluorescent reporter dye at the 5′ end, and a TAMRA quencher dye at the 3′ end. Each reaction consisted of 2× Universal Master Mix II (Applied Biosystems), primers, labeled probes, and 6.5 ng cDNA in a total volume of 40 μL. Reactions were initially denatured at 95 °C for 10 min, and then amplified over 40 cycles at 95 °C for 15 s and 60 °C for 1 min using a Real-Time PCR system 7500 (Applied Biosystems). Fluorescence was measured during the elongation step. Data are reported as fold induction relative to untransfected cells.

STAT6 phosphorylation

STAT6 phosphorylation was quantified using PathScan phospho-STAT6 sandwich ELISA kit (Cell Signaling Technology Japan, Tokyo, Japan). Briefly, cells were incubated with or without 10 ng/mL IL-13 for 10 or 20 min, harvested with a scraper, and lysed in lysis buffer. Lysates were added to 96-well microtiter plates coated with antibodies specific for STAT6 phosphorylated at Tyr641. After incubation at 37 °C for 2 h, wells were washed four times with wash buffer, incubated at 37 °C for 1 h with an antibody against phosphorylated STAT6, and washed another four times with wash buffer. Wells were then probed at 37 °C for 30 min with a secondary antibody conjugated to horseradish peroxidase, washed four times with wash buffer, and reacted at 37 °C for 30 min with tetramethylbenzidine. The reaction was terminated with stop solution, and absorbance at 450 nm was measured using the ELISA plate reader Glomax-Multi Detection System (Promega). Data are reported as optical density.

Stability of CCL11 and CCL26 mRNA

To determine the effect of miR-155 overexpression on the stability of CCL11 and CCL26 mRNA, we treated BEAS-2B cells with

10 ng/mL IL-13 for 24 h after transfection with negative control (miR-control) or miR-155 mimic. Cells were then either harvested or treated with transcription inhibitor actinomycin D (3 μg/mL) for indicated time, and then subjected to real-time PCR to determine the decay of the chemokines' mRNA.

Statistical analysis

Data are reported as mean ± standard error of the mean (SEM). Data were analyzed in Stat-View 5.0 (Abacus Concepts, Inc., Berkeley, CA, USA) by analysis of variance with Fisher's protected least significant difference test.

Results

Downregulation or overexpression of miR-155 in bronchial epithelial cells

miR-155 expression was significantly down regulated in BEAS-2B bronchial epithelial cells transfected with miR-155 inhibitor than in cells transfected with negative control (miR-control) ($^*p < 0.05$, Fig. 1A), but was significantly upregulated in cells transfected with miR-155 mimic than in cells transfected with negative control (miR-control) ($^*p < 0.05$, Fig. 1B). Transfection with miR-control did not change the expression level of miR-155 (NS means not-significance).

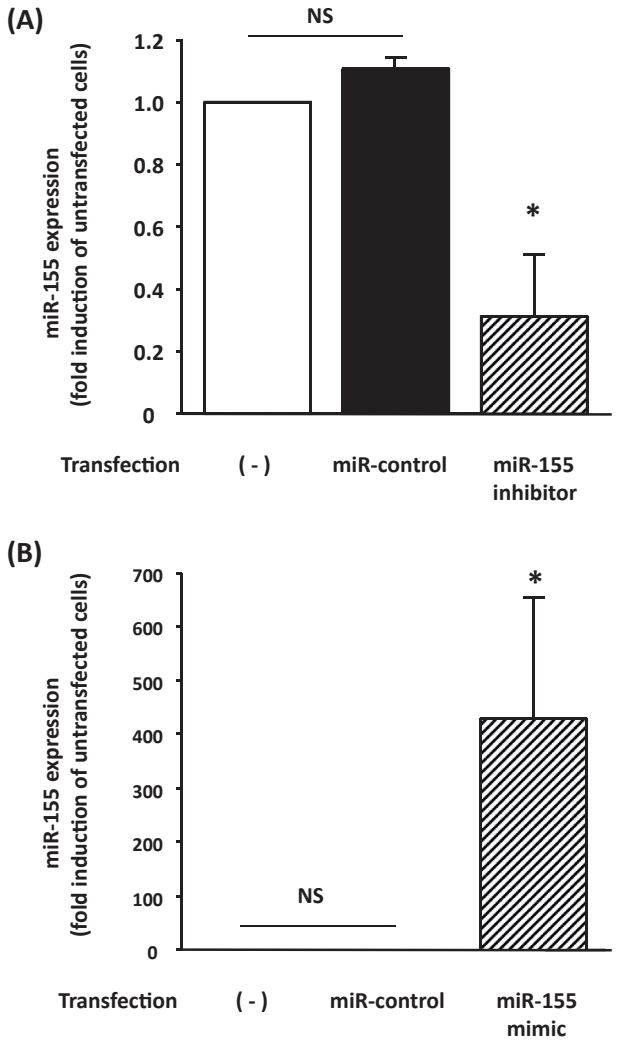

Fig. 1. Expression of miR-155 in BEAS-2B bronchial epithelial cells transfected with 25 nM miR-155 inhibitor **(A)** and mimic **(B)**, as measured by real-time PCR. A non-specific miRNA was used as negative control (miR-control). Data are mean ± SEM of three independent experiments. $^*p < 0.05$ vs. cells transfected with miR-control. NS means not-significance.

CCL11 and CCL26 secretion in cells stimulated with IL-13

As previously reported,[12] CCL11 secretion increased within 24 h ($^*p < 0.05$) in untransfected cells treated with TNF-α and IL-13, but not in untransfected cells treated with dsRNA (Fig. 2A). Similar results were obtained in cells transfected with miR-negative control or miR-155 inhibitor. However, miR-155 overexpression via miR-155 mimic significantly blocked the increase in CCL11 secretion due to IL-13, with $^{**}p < 0.05$ when compared to the cells transfected with miR-control and stimulated with IL-13. miR-155

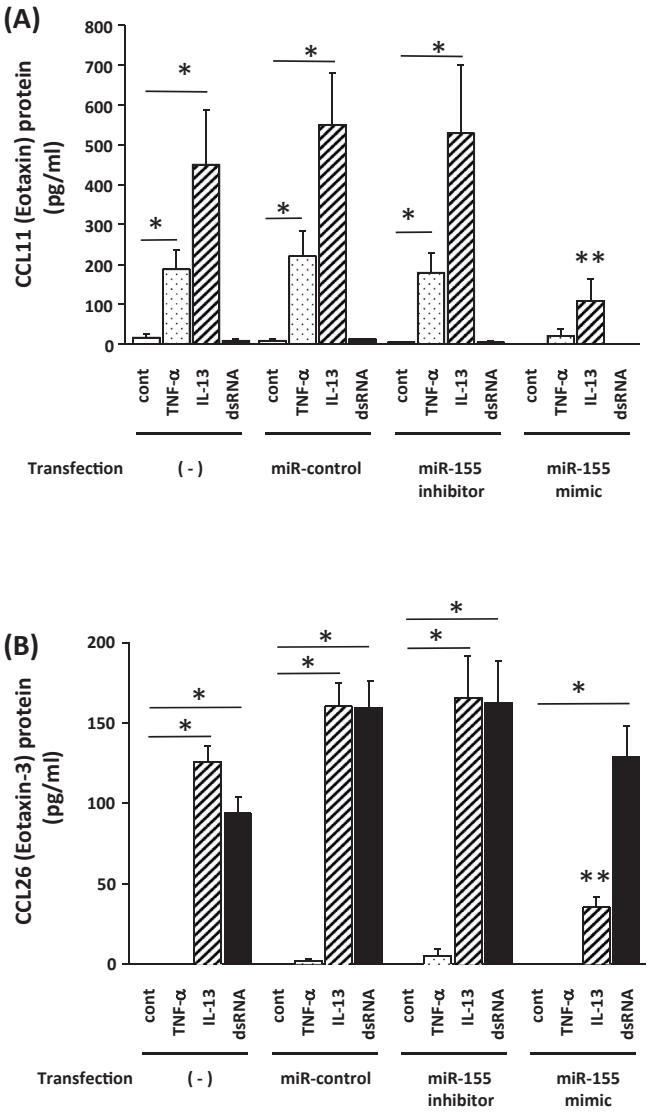

Fig. 2. Secretion of the eosinophilic chemokines CCL11/Eotaxin **(A)** and CCL26/Eotaxin-3 **(B)** by BEAS-2B bronchial epithelial cells. Cells transfected with or without miR-155 mimic, inhibitor, or negative control (miR-control) were stimulated for 24 h with 10 ng/mL IL-13, 10 ng/mL TNF-α, or 50 μg/mL dsRNA poly IC. Cell culture supernatants were analyzed by ELISA. Data are mean ± SEM of three independent experiments. $^*p < 0.05$ vs. unstimulated cells (cont) with same situation; $^{**}p < 0.05$ vs. cells transfected with miR-cont and exposed to the same stimulant.

overexpression seemed to block CCL11 production in cells treated with TNF-α, but not to a statistically significant extent. In untransfected cells, IL-13 and dsRNA stimulated secretion of CCL26 within 24 h (*$p < 0.05$, Fig. 2B). However, TNF-α stimulated CCL26 secretion only slightly, and not to a statistically significant extent. The same effects were observed in cells transfected with miR-155 inhibitor and negative control. However, production of CCL26 due to IL-13 was significantly blocked in cells overexpressing miR-155 via miR-155 mimic, with **$p < 0.05$ relative to cells transfected with miR-control and stimulated with IL-13. Notably, production of CCL26 due to dsRNA was independent of miR-155 overexpression.

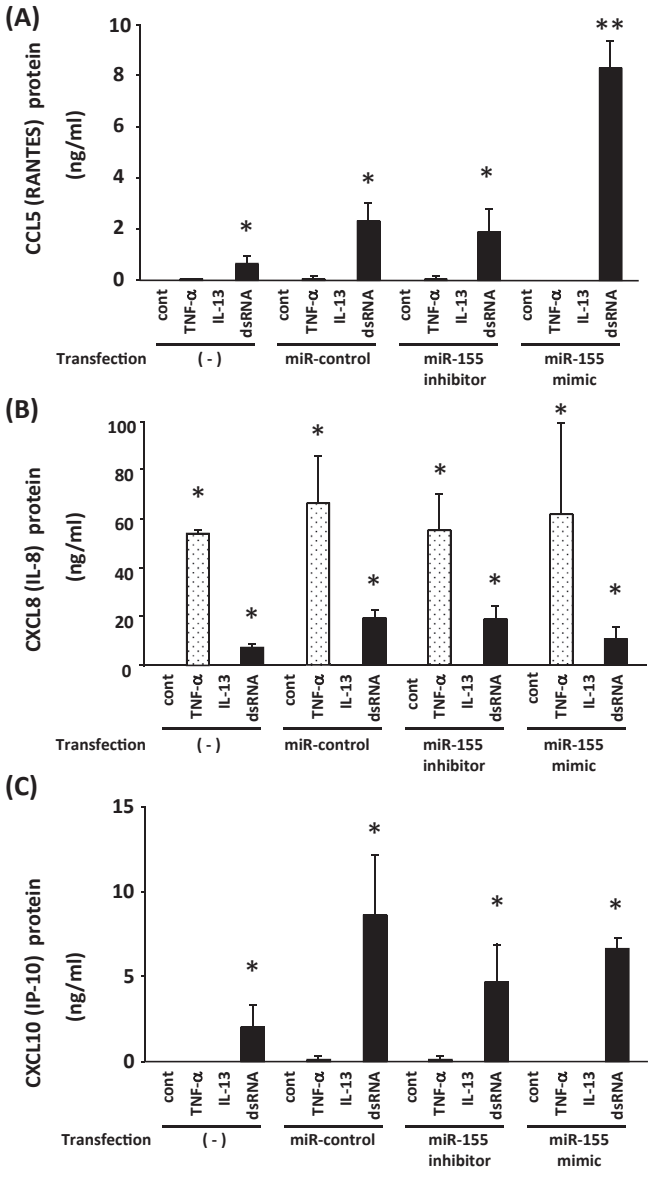

Fig. 3. Production of the chemokines CCL5/RANTES **(A)**, CXCL8/IL-8 **(B)**, and CXCL10/IP-10 **(C)** by BEAS-2B bronchial epithelial cells transfected with or without miR-155 mimic, inhibitor, or negative control (miR-control), and stimulated for 24 h with 10 ng/mL IL-13, 10 ng/mL TNF-α, or 50 μg/mL dsRNA poly IC. Chemokines secreted into the cell culture supernatants were measured by ELISA. Data are mean ± SEM of three independent experiments. *$p < 0.05$ vs. unstimulated cells (cont) with same situation; **$p < 0.05$ vs. cells transfected with miR-control and exposed to the same stimulant.

Effect of miR-155 on secretion of CCL5, CXCL8, and CXC10

In untransfected cells, TNF-α stimulated production of CXCL8 (Fig. 3B), while dsRNA increased production of CCL5 (Fig. 3A), CXCL8 (Fig. 3B), and CXCL10 (Fig. 3C) 24 h after exposure (*$p < 0.05$), as previously reported.[14] However, IL-13 did not increase secretion. Notably, miR-155 overexpression via miR-155 mimic did not inhibit secretion, indicating that the miRNA specifically blocks CCL11 and CCL26 production due to IL-13. On the other hand, negative control (miR-control), mimic, and inhibitor miRNAs seemed to relatively enhance chemokine production in response to dsRNA.

CCL11 and CCL26 mRNA

We then investigated the mechanisms underlying the ability of miR-155 to block CCL11 and CCL26 production due to IL-13. In the cells transfected with negative control miRNA, the abundance of CCL11 (Fig. 4A) and CCL26 mRNA (Fig. 4B) increased 24 h after stimulation with IL-13 (*$p < 0.05$). These effects were blocked by

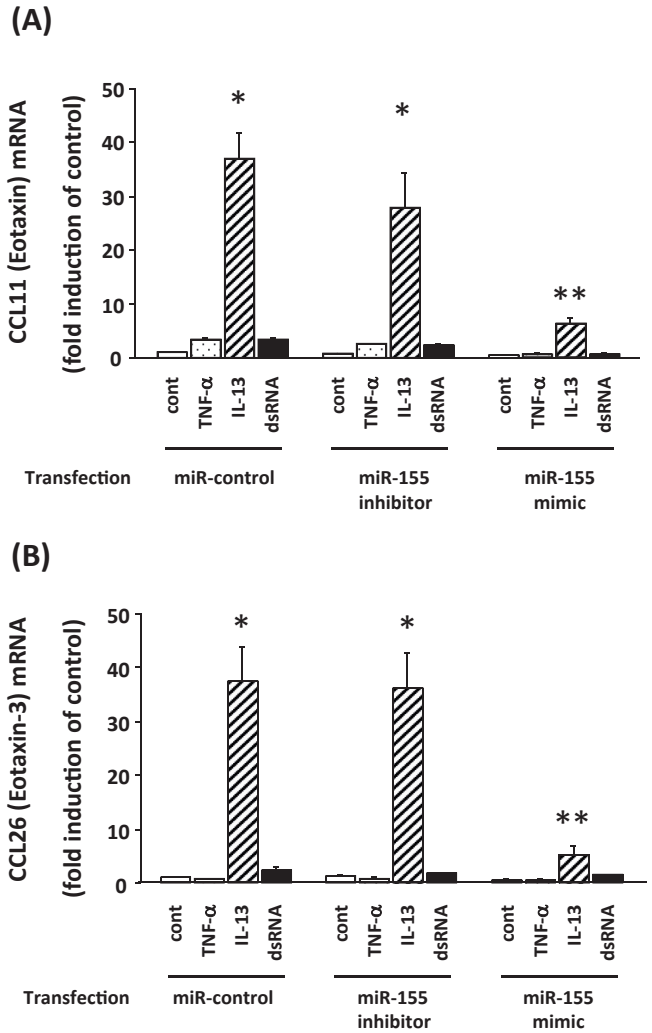

Fig. 4. Abundance of CCL11/Eotaxin **(A)** and CCL26/Eotaxin-3 **(B)** mRNA in BEAS-2B bronchial epithelial cells. Cells were transfected with miR-155 mimic, inhibitor, or negative control (miR-control), stimulated for 24 h with 10 ng/mL IL-13, 10 ng/mL TNF-α, or 50 μg/mL dsRNA poly IC, and analyzed by real-time PCR. Data are mean ± SEM of three independent experiments. *$p < 0.05$ vs. unstimulated cells (cont); **$p < 0.05$ vs. cells transfected with negative control and exposed to the same stimulant.

overexpression of miR-155 cells via miR-155 mimic, with $^{**}p < 0.05$ relative to IL-13-stimulated cells transfected with negative control miRNA (miR-control), even though the 3′UTR in these chemokines do not contain the consensus binding site for miR-155.

Effects of miR-155 overexpression on IL-13 signaling

We then hypothesized that miR-155 might regulate IL-13 signaling upstream of CCL11 or CCL26 mRNA synthesis. We found that BEAS-2B bronchial epithelial cells constitutively expressed IL-13 receptor alpha 1, and expression was not suppressed by miR-155 overexpression via miR-155 mimic (Fig. 5A), in contrast to published data in macrophages.[7] We also analyzed the effect of miR-155 overexpression on IL-13 signaling downstream of the IL-13 receptor. As expected, IL-13 induced phosphorylation of STAT6 (Fig. 5B), a key transcription factor for that regulates CCL11 and CCL26 expression.[12,18,19] We found that miR-155 overexpression did not interfere with STAT6 phosphorylation in cells exposed to IL-13.

Effect of miR-155 overexpression on the mRNA stability

Results of real-time PCR for CCL11 (Fig. 6A) and CCL26 (Fig. 6B) showed stability of mRNA for these chemokines decreased after adding actinomycin D at 4 h ($^{*}p < 0.05$ vs. cells at time 0 h). We hypothesized that overexpression of miR-155 might accelerate the decay of chemokines' mRNA, if miR-155 directly binds and interferes with mRNA for CCL11 or CCL26. However, level of decay of chemokines' mRNA were not different between cells transfected with negative control (miR-control) and the cells transfected with miR-155 mimic. These data indicate that the effect of miR-155 overexpression may not be derived from direct interference with chemokines' mRNA.

Discussion

In this study, we demonstrated that in BEAS-2B bronchial epithelial cells, miR-155 blocks secretion of CCL11 and CCL26 due to the Th2 cytokine IL-13. However, miR-155 did not block dsRNA or TNFα-induced secretion of other chemokines such as CCL5, CXCL8, and CXCL10. Accordingly, miR-155 downregulated CCL11 and CCL26 mRNA expression, as measured by real-time PCR. Nevertheless, downregulation of miR-155 did not stimulate expression of CCL11 and CCL26, in contrast to expectation. Finally, miR-155 overexpression did not suppress expression of the IL-13 receptor or alter the phosphorylation of its downstream target STAT6.

The effects of miR-155 overexpression did not seem to be experimental artifacts, as non-specific control oligonucleotides did not block IL-13-induced expression of CCL11 and CCL26. In addition, the effects of miR-155 also seemed to be dose-dependent over 5–50 nM in preliminary experiments (data not shown). Further, we

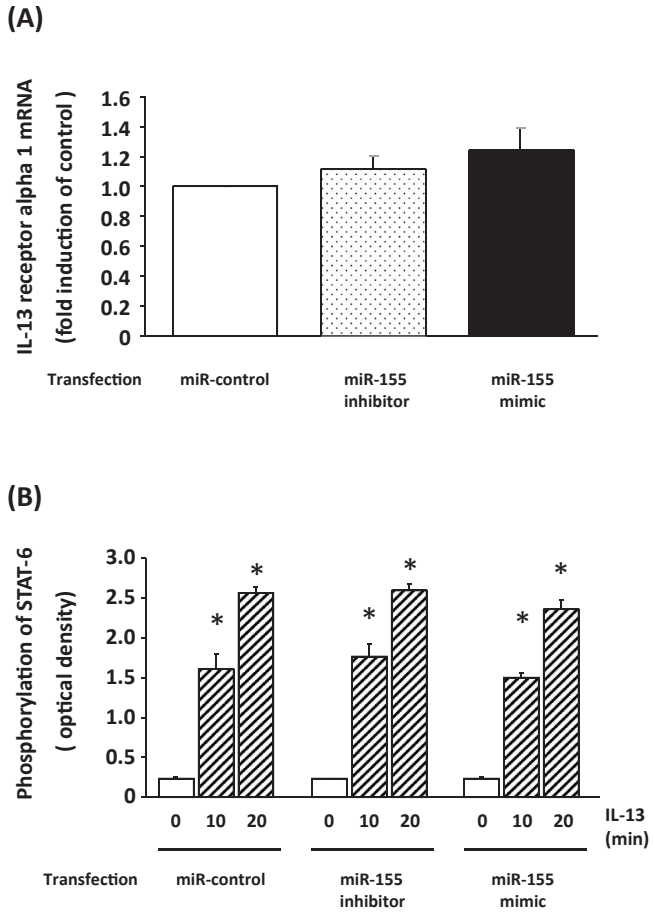

Fig. 5. Expression IL-13 receptor alpha 1, as measured by real-time PCR, in BEAS-2B bronchial epithelial cells overexpressing miR-155 via miR-155 mimic **(A)**. STAT6 phosphorylation, as measured by ELISA, in BEAS-2B cells transfected with indicated miRNAs, and stimulated with 10 ng/mL IL-13 for 10 or 20 min **(B)**. Data are mean ± SEM of three independent experiments. $^{*}p < 0.05$ vs. unstimulated cells (time 0 h) with same situation.

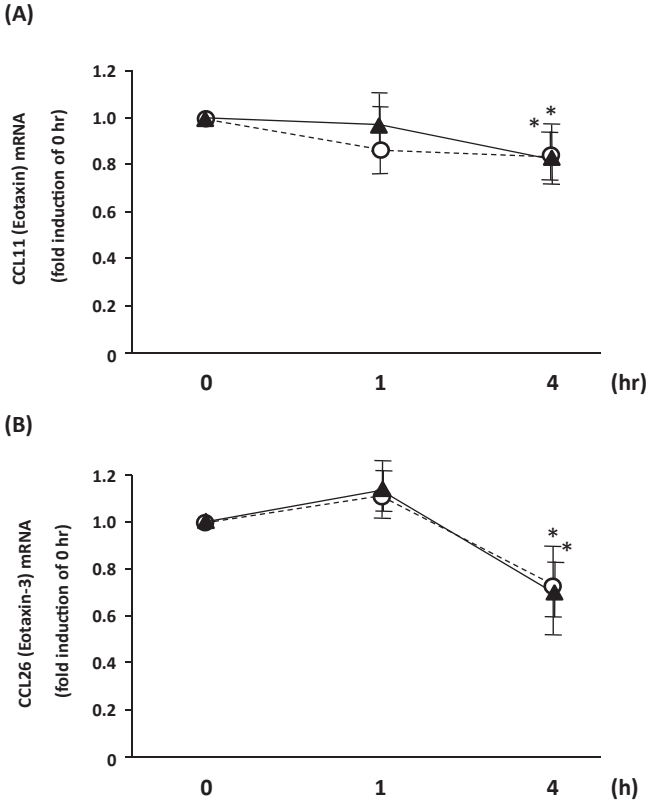

Fig. 6. Effect of overexpression of miR-155 on the decay of mRNA for CCL11/Eotaxin **(A)** and CCL26/Eotaxin-3 **(B)** in BEAS-2B cells. Cells were transfected with negative control (miR-control) (open circle) or miR-155 mimic (closed triangle) and treated with the transcription inhibitor actinomycin D (3 μg/mL) for the indicated time after pretreatment with 10 ng/mL IL-13 for 24 h. Quantification of mRNA was performed with real-time PCR and the data were expressed as fold induction of cells time 0 h (not treated with actinomycin D). Data are representatives of three independent experiments. $^{*}p < 0.05$ vs. cells not treated with actinomycin D (time 0 h) with same situation.

also confirmed that several miRNAs, including miR-21 and miR-146, did not interfere with chemokine expression (data not shown). Finally, we verified that miR-155 overexpression did not impact cell viability (data not shown).

We need further studies to clarify the reason why miR-155 inhibitor failed to show any effect on the chemokines' expression, however we hypothesize that the degree of inhibitory effect and other miRNAs may be involved. Martinez-Nunez RT *et al.* have been reported that downregulation of miR-155 did not alter the expression of inflammatory cytokines in epithelial cells, but downregulation of multiple miRNAs, such as miR-155, miR18a, miR-27a and miR-128, resulted in the upregulation of IL-6 and IL-8.[5] These data suggest that some miRNA compensate other miRNA's function and multiple miRNAs co-operatively regulate target mRNA expression. In our study, we hypothesize that function of downregulation of miR-155 may be compensated by other miRNA. The degree of downregulation of miR-155 expression level seemed to be lower than that of miR-155 overexpression. Remarkable level of overexpressed miR-155 may be necessary for getting its function to downregulate the expression of CCL11 and CCL26. Level of miR-155 constitutively expressed in BEAS-2B cells might not be enough to suppress the expression of CCL11 or CCL26.

We used *TargetScan* for bioinformatic analysis to analyze whether miR-155 can bind 3′UTR of CCL11 or CCL26. It is the web server and predicts the presence of 8 or 7mer sites in the target mRNA that match seed region in each miRNA. The seed region is essential for the binding of miRNA to the target mRNA and should be complementary with the sequence of target 3′UTR.[20] It is mostly situated at positions 2–8 of the miRNA 5'-end. We analyzed the 3′UTR of CCL11 and CCL26 mRNA and could not find conserved binding site for miR-155 in these chemokines. Accordingly, our data suggested that miR-155 overexpression did not interfere with the stability of CCL11 and CCL26 mRNA. Therefore, we hypothesized that the inhibitory effects of miR-155 on chemokine production may be indirect, we need further studies to confirm these hypothesis using reporter plasmids containing 3′UTR of these chemokines however.

Thus, we tested whether miR-155 might suppress expression of IL-13 receptor alpha 1 in BEAS-2B cells. However, we found that miR-155 does not suppress IL-13 receptor alpha 1 in these cells, as it does in macrophages.[7] This discrepancy may be due to differences between cell types. It has been documented that role of miRNAs is sometimes different between cell types.[21] For example, some investigators have reported that miR-155 targets transcription factors Smad 2 and Smad 5, and directly suppress these factors' expression in macrophage.[22,23] On the hand, Yao R. *et al.* has reported that miR-155 could not directly inhibit the expression of Smad 2 or Smad 5.[24] These mechanisms have not been clarified in details, but the investigators discussed that many kinds of miRNAs regulate the target genes and miRNA tends to inhibit different transcripts in a cell-specific manner. In addition to the difference of cell type, different protocol and system of experiments may influence the results.[21]

We also analyzed STAT6 phosphorylation, a key transcription factor that stimulates transcription of CCL11 and CCL26 in response to IL-13. We found that miR-155 overexpression also did not affect STAT6 phosphorylation in BEAS-2B cells.

Thus, further research is necessary to elucidate the mechanisms underlying the ability miR-155 to block the effects of IL-13 on expression of CCL11 and CCL26. We now hypothesize that the transcription factor PU.1 may be involved. PU.1 stimulates transcription of target genes via STAT6, and expression of PU.1 itself is repressed via a binding site for miR-155.[25,26] Consequently, miR-155 overexpression may block the effects of IL-13 if PU.1 is involved in the IL-13 pathway of CCL11 or CCL26 induction. In our results, miR-155 overexpression did not inhibit the expression of CCL11 or CCL26 stimulated with TNF-α. We hypothesize that these mechanisms may be also explained by the role of PU.1, because PU.1 may not interact with NF-κB, a responsible transcription factor for TNF-α signaling.

We showed the differential regulation of chemokine expression by cytokines or dsRNA. We have previously compared the effects of stimulation with dsRNA on the expression of chemokines in airway epithelial cells.[14] The effects were grater on the expression of CCL5 and CXCL10 than CXCL8 or CCL26. It did not stimulate CCL11 expression. We hypothesized that structure of chemokine's promoter region may be responsible for the activation of chemokine expression through TLR3 signaling. CCL5 and CXCL10 contain the response element for two important transcription factors, nuclear factor-kappa B (NF-κB) and interferon regulatory factor 3 (IRF-3), on the other hand, CXCL8 or CCL26 contains the response element for only NF-κB. Cooperative activation with both NF-κB and IRF-3 may be necessary for significant stimulation of the chemokine expression. IL-13 increased the expression of CCL11 and CCL26, while it did not stimulate other chemokines. Consensus sequence for STAT6 binding is TTCN3/4GAA.[18] These putative sequence for STAT6 exist in the promoter region of these chemokines, however there are no report to show binding of STAT6 to theses chemokines' promoter in our knowledge.[27–29] These chemokines are known to be less responsive to Th2 cytokine. Some investigators have also reported that CXCL10 expression was independent to STAT6 using STAT6-deficient mice.[30] In addition, we could not find any consensus binding site of miR-155 in the 3′UTR region of CCL5, CXCL8, or CXCL10 using *TargetScan*. Taken together, IL-13-STAT6 signaling may not be involved in the stimulation of CCL5, CXCL8 or CXCL10 expression. If miR-155-PU.1 interaction would be specific for STAT6 function, miR-155 may not interfere with the expression of CCL5, CXCL8 or CXCL10.

Newly discovered cytokines derived from bronchial epithelial cells, such as thymic stromal lymphopoietin, IL-33 and IL-25, would be very important in the initiation of Th2-type response in asthma.[31] These cytokines may contribute to stimulate CCL11 or CCL26 through production of Th2 cytokine and subsequent prolonged eosinophilic inflammation in airway. To elucidate the role of miR-155 in the pathogenesis of asthma, we need further studies to examine whether miR-155 influence some effects on the production of these new cytokines.

In any case, the results indicate that miR-155 may negatively regulate inflammatory factors related to Th2-type immunity, in accordance with previous reports. Indeed, decreased expression of miR-155 in the epithelium may indicate a lack of anti-inflammatory activity in the airway, and contribute to the development of asthma.[4–6]

In summary, our results demonstrate that overexpression of miR-155 in BEAS-2B bronchial epithelial cells significantly suppresses IL-13-induced production of CCL11 and CCL26, which are chemoattractants for eosinophils. While further studies in primary epithelial cells are required to elucidate the underlying mechanism in detail, our data provide new opportunities to exploit miR-155 in asthma therapy.

Acknowledgments

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

All authors managed the study. SM and FK designed the study and are responsible for grants. SM performed most experiments, and wrote the manuscript. YO, AS, DI, YK, TF, HTak, YY, and HTat collected data. GI, YT, AW, EK, MH, FY, KK, and HS analyzed and interpreted data.

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